Use of BCG vaccine as a preventive measure for COVID-19 in health care workers

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Contents:

RESEARCHERS:

Fernanda Carvalho de Q. Mello; MD, MSc, PhD; CPF 916357567 15

Afrânio Lineu Kritski; MD, MSc, PhD; CPF 299576569 53

José Roberto Lapa e Silva; MD, MSc, PhD; CPF 127025017 53

Elisangela Costa da Silva; MSc. PhD; CPF 016548077 70

Rafael Galliez; MD, MSc. PhD; CPF 082542837 88

Maria Armanda Monteiro da Silva Vieira; MD, MSc, PhD; CPF 707915627 00

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Antonio Guilherme Fonseca Pacheco; MD, MSc, PhD; CPF 012999877 02

Martha Maria Oliveira; MSc, PhD; CPF 042874657 84

Guilherme Loureiro Werneck; MD, MSc, PhD, CPF 770037137 72

Rogério Lopes Rufino Alves; MD, MSc, PhD; CPF 821459627 00

Luís Cristóvão de Moraes Sobrino Porto, MD, MSc, PhD; CPF: 429488357 04

Hanna Silva Condelo, MSc; PfC: 135268117 09

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Robson de Souza Leão; MSc, PhD; CPF 034374517 82

Luciana Silva Rodrigues; MSc, PhD; CPF 075691427 21

Janaína Aparecida of Medeiros Leung; MSc; CPF 160954918 00

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Roberto de Andrade Medronho; MD, MSc, PhD; CPF 508401427 49

Terezinha Marta Pereira Pinto Castineiras; MD, MSc, PhD; CPF 982013527 34

PARTICIPATING INSTITUTIONS:

1. Clementino Fraga Filho University Hospital - Thorax Diseases Institute, Federal University of Rio de Janeiro - Rio de Janeiro/RJ
2. Pedro Ernesto University Hospital - Piquet Carneiro Polyclinic, State University of Rio de Janeiro - Rio de Janeiro/RJ
3. Central Hospital of the Fire Department of RJ Aristarcho Pessoa - Rio de Janeiro/RJ
4. Dr. Francisco Moran Municipal Hospital - Barueri/SP

SUBSCRIPTION PAGE (RESEARCHER - SPONSOR)

I inform you that this Protocol has been revised and approved.

We will supervise and coordinate the clinical trial, conducted in 4 centers in Brazil as described and ensure compliance with Good Clinical Practices (BPC) and Good Laboratory Practices (BPL), the principles described in the Helsinki Declaration and the applicable regulatory requirements.

Name: Fernanda Carvalho de Queiroz Mello

Institution: Thorax Diseases Institute - Federal University of Rio de Janeiro

Signature:

_____

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day/month/year

STATEMENT BY THE PRINCIPAL INVESTIGATOR

By signing this page, I agree to conduct the study in accordance with all current Brazilian regulations regarding applicable clinical research and international guidelines, as stated in the protocol and other information provided to me.

I will ensure that the requirements for obtaining review and approval from the Research Ethics Committee (CEP) are met. I will promptly report to the Ethics Committee any and all changes in research activities covered by this protocol.

I certify that the individuals involved with this study have completed training in Good Clinical Practices (BPC) in the last 3 years and, if applicable, training in the protection of human beings.

I understand that all information obtained during the conduct of the study regarding the health status of individuals will be considered confidential. No individual name or personally identifiable information may be disclosed. All subject data will be anonymized and identified by numbers assigned in all Case Report Forms and laboratory samples.

I will maintain the confidentiality of this protocol and all other related investigative materials. The information obtained from the study protocol cannot be disclosed or discussed with third parties without the express consent of the researcher – Dr. Fernanda Carvalho de Queiroz Mello.

Full Name of the Principal Investigator:

Fernanda Carvalho de Queiroz Mello

Signature:

_____

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day / month / year

Summary:

The disease promoted by coronavirus (COVID-19) is caused by SARS-CoV2, being the first cases identified in December 2019 in China after exposure to the animal market in Wuhan city, China (reviewed by Adhikari et al, 2020). From the first case to the present day, the COVID-19 epidemic has been identified in 185 countries, with the notification of 2,666,154 cases and 186,144 deaths (Source: COVID Visualizer, 23Apr2020). In Brazil, more than 45,757 cases and 2,906 confirmed deaths by COVID-19 have been confirmed (Source: COVID Visualizer, 23Abr2020). In our country, to date, testing for COVID-19 occurs only in severe cases and few centers offer the service to health care workers, a population at high risk of infection. BCG is a vaccine produced from a live attenuated strain derived from a *Mycobacterium bovis* isolate and is widely used worldwide as a tuberculosis (TB) vaccine, but there are studies demonstrating non-specific immunotherapeutic mechanisms of this vaccine that signal a possible relationship with the lowest morbidity and mortality associated with COVID-19 infections worldwide. The present study aims to analyze the role of BCG in the prevention of SARS-CoV-2 infection and also in the occurrence of severe forms of COVID-19 in addition to evaluating the immune response mediated by this vaccine in voluntary health care workers.

INTRODUCTION:

The disease promoted by coronavirus (COVID-19) is caused by SARS-Cov2, being the first cases identified in December 2019 in China after exposure to the animal market in Wuhan city, China (reviewed by Adhikari et al,2020).

From the first case to the present day, the COVID-19 epidemic has been identified in 185 countries, with the notification of 2,666,154 cases and 186,144 deaths (Source: COVID Visualizer, 23Apr2020). In Brazil, more than 45,757 cases and 2,906 confirmed deaths by COVID-19 have been confirmed (Source: COVID Visualizer, 23Abr2020). In our country, to date, testing for COVID-19 occurs only in severe cases and few centers offer the service to health professionals, a population at high risk of infection. Thus, the data in Brazil present a high of underreporting. According to the Federal University of Rio de Janeiro (UFRJ) Technical Note, it is estimated that the cases reported in Brazil represent approximately only 11% of all (Kritski *et al*, 2020).

It is known that the impact of the disease differs between countries. These differences are attributed to the different cultural norms, mitigation efforts, available health infrastructure and socio-economic level. Recently, Miller *et al* (2020) proposed, through an ecological study, that countries that continued with the use of vaccination with *Bacillus Calmette Guérin* (BCG) after birth have a lower occurrence of COVID-19.

BCG is a vaccine produced from a live attenuated strain derived from an isolate of *Mycobacterium bovis* and is widely used worldwide as a tuberculosis (TB) vaccine, and countries such as Portugal, Japan and China adopt a universal BCG vaccination policy in newborns. Other countries such as Italy, the Netherlands, Spain, France, Switzerland and the United States of America have discontinued or failed to adopt universal vaccination policies due to the low risk, when compared to countries with high prevalence of the disease, of developing *Mycobacterium tuberculosis* (MTB) infections, and also due to the variable efficacy in the prevention of tuberculosis in adults (Miller *et al*,2020).

Interestingly, shortly after its introduction in the 1920s, epidemiological studies showed that the BCG vaccine reduced infant mortality regardless of its effect on TB (reviewed by Shan *et al*, 2009). In several observational studies in West Africa, a 50% reduction in the overall mortality of children vaccinated with BCG was observed, an effect too large to be explained by the protection against tuberculosis only (Garly *et al,*2003; Roth *et al*,2005). More recently, these findings have been validated in controlled trials (Aaby *et al*,2011). Therefore, the reduction in mortality in infants by BCG seems to be due to the

induction of protection against unrelated infectious agents. These beneficial effects have been called "heterologous" or "non-specific" effects.

There are two basic mechanisms on the effect of BCG vaccine on heterologous responses:

1) BCG induces lymphocytic action, resulting in more efficient immune reactions related to secondary responses not related to infectious agents (Goodridge *et al*, 2016). For example, mice are protected by BCG vaccination against *vaccinia* virus infection by increasing the production of IFN-γ by T cells CD4+ (Mathurin *et al,*2009). The recurrent lymphocytic responses may also involve the activation of CD4+ and T memory cells that are specific to non-target antigens, thus modulating T_H1 and T_H17 responses to secondary non-mycobacterial infections. In healthy volunteer humans, BCG vaccination increased non-specific responses of T_H1 and T_H17 subpopulations for at least 1 year after vaccination (Kleinnijenhuis *et al,*2014). In addition, in patients with recurrent respiratory papillomatosis, which is caused by human papillomavirus in 90% of cases, adjuvant therapy with BCG restored the efficiency of antiviral T-cell responseby stimulating the production of T_H1 cytokines and induction of $_{REG}$ T cells.

2) Less than a decade ago, only B and T lymphocytes were considered capable of constructing memory responses. However, in recent studies it has been shown that the functional program of innate immune cells is altered in certain infections or vaccinations, resulting in increased immunity when cells encounter a secondary stimulus (Netea *et al*, 2016). The induction of a non-specific memory in innate immune cells is known as "trained immunity" and is mediated by epigenetic and metabolic remodeling (Netea *et al*,2016). Evidence suggests that the key mechanism by which BCG induces its non-specific effects is probably through the induction of immune memory in innate immune cells, especially in natural killer cells (NK), monocytes and macrophages, and not by means of adaptive immune mechanisms based on T cells and B cells.

In mice with severe combined immunodeficiency, the BCG vaccine provided protection against a non-mycobacterial secondary challenge, confirming the importance of innate immune cells in mediating this effect (Arts *et al*, 2018). In healthy human volunteers, BCG vaccination improved the production of pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α in peripheral blood mononuclear cells (PBMC) for up to 3 months after vaccination on in vitro stimulation with unrelated pathogens (Arts *et al,*2018). This response was associated with an increase in CD11b,

TLR4 and CD14 activation markers and epigenetic reprogramming of human monocytes at gene-promoting sites for pro-inflammatory cytokines. In a randomized BCG study, volunteer humans were vaccinated with the yellow fever vaccine, an attenuated virus vaccine. In this study, a significant reduction in viremia was observed after 1 month or placebo in volunteers vaccinated with BCG when compared to volunteers who received placebo. And this effect was correlated with epigenetic mutations induced by BCG in monocytes (Arts *et al*,2018). BCG induced higher production of IL-1β during viral infections, rather than the production of IFN-γ by lymphocytes and NK cells. The protective role of IL-1β is related to recent findings that suggest a crucial role of this interleukin in antiviral immunity (Rathinam *et al*,2010).

Finally, several authors have discussed the role of BCG in inducing the IFN-I pathway in circulating monocytes, inducing the production of IFN-α and -β, cytokines known to be important in the control of viral replication (Moorlag *et al,*2019). Modulation of a T_H1 response, with activation of monocytes/macrophages, may help reduce the severe responses associated with COVID-19, as several authors point out that inflammatory responses associated with severe forms of Severe Acute Respiratory Syndrome (ARDS) are associated with neutrophil-mediated responses, T_H17 profiles, pro-inflammatory cytokines such as IL-6, in an event called cytokine storm (reviewed by Jiang *et al,*2019). To build an antiviral response, the ininate immune system recognizes molecular structures that are produced by virus invasion, called pathogen-associated molecular patterns (PAMPs). For RNA viruses such as coronavirus, it is known that the PAMPs found in virus replication in the form of viral genomic ssRNA or double-tapR RNA are recognized by the endosome receptors of TLR8 and TLR7 RNA in ssRNA and by the cytosolic RNA sensor, RIG (rethyroid gene)/MDA5 (gene 5 associated with melanoma differentiation) (Li *et al*, 2020; de Wit *et al*,2016; Channappanavar *et al*,2017). This recognition leads to the activation of several signaling routes. Transcription factors such as nuclear factor κB (NF-κB), activating protein 1 (AP-1), interferon response factor 3 and 7 (IRF3 and IRF7). NF-κB and AP-1 stimulate the expression of genes encoding several molecules necessary for inflammatory responses, including inflammatory cytokines (e.g., TNF-α and IL-1) and chemokines (CCL2 and CXCL8). IRF3 and IRF7 promote the production of type I interferon (IFN-α and IFN-β), important for innate antiviral immune responses capable of suppressing replication and viral dissemination at an early stage (Deng *et al,*2019; Yang *et al*,2015). This process can cause complications such as exhaustion, weakness and cough in patients (Kindler *et al*,2016).

The important point in the current pandemic scenario by COVID-19 is that, for SARS-CoV-2, the response to viral infection by IFN type I is suppressed (reviewed by Rokni *et al*, 2020), because hospitalized patients with severe COVID-19 have high levels of pro-inflammatory cytokines, including IL-2, IL-7, G-CSF, IP-10, MCP-1, MIP-1α, and TNF-α. This phenomenon known as "cytokine storm" or cytokine release syndrome becomes an important factor in the pathogenesis of COVID-19 (Wongi et al, 2004; Conti *et al*,2020). New strategies should be researched to analyze and improve the individual's response against SARS-CoV2 infection.

Thus, it is understood that non-specific immunotherapeutic mechanisms of BCG vaccine in the light of current epidemiological data, indicate a possible relationship between the existence of universal BCG vaccine policies and the lower morbidity and mortality associated with COVID-19 infections worldwide.

JUSTIFICATION:

Considering the above, the present project aims to study the effect of BCG vaccine on health care workers of reference hospitals for the care of COVID-19.

This project presents an innovative proposal, addressing a BCG vaccine with known efficacy against severe TB in childhood and reliable for the use of adjuvant therapy in patients with malignant bladder cancer.

BCG is an easily accessible vaccine, widely used by the universal system of health care in Brazil (SUS), in newborn children, thus facilitating its incorporation into a new preventive proposal for COVID-19. In addition, the selection of health care workers will be based on diagnostic screening and confirmatory tests of COVID-19.

It is intended to analyze the role of BCG in the prevention of infection and also in the occurrence of severe forms of COVID-19 in addition to evaluating the immune response mediated by this vaccine in voluntary health professionals.

OBJECTIVES:

In the 12 months period, the study aims to analyze the effect of BCG as a protective factor for COVID-19 in health care workers, with a greater efficiency in the prevention of subjects at high risk of infection and evolution to severe forms of the disease, as well as to evaluate the specific and non-specific immune responses to SARS-CoV-2 in vaccinated and unvaccinated professionals who evolved with COVID-19 or not.

Primary Objectives:

1. Assess the impact of BCG vaccine administration on the cumulative incidence of SARS-CoV-2 infection
2. Assess the impact of BCG vaccine administration on the cumulative incidence of severe forms of COVID-19
3. Evaluate the BCG vaccine-mediated immune response in volunteer health care workers

Secondary Objectives:

In groups receiving BCG or placebo, infected and not infected with SARS-CoV-2:

- Epidemiological:

1. Estimate whether BCG vaccination compared to placebo prolongs the time to the first respiratory disease proven by SARS-CoV-2;
2. Estimate whether BCG vaccination compared with placebo reduces the proportion of symptoms associated with COVID-19 (fever or at least one sign/symptom of respiratory disease);
3. Estimate whether BCG vaccination compared to placebo reduces absenteism (days of absence) in health care workers;
4. Analyze the safety of BCG vaccination in adult health care workers;

- Laboratories:

5. Describe the inflammatory profile by dosing neutrophils, platelets, cit-H3, alpha 1 antitrypsin (α1AT), galectin-2, ferritin, transferrin-R, glycated hemoglobin (HbA1c),

C-reactive protein (CRP), D-dummer, LDH, albumin and erythrocyte sedimentation rate (ESR);

6. Describe the profile of inflammatory cytokines identified in the BCG and placebo groups, comparing to the protective events to SARS-CoV-2;
7. Describe the epigenetic alterations associated with monocytes and neutrophils related to the protection of COVID-19;
8. Characterize the cell population (T, B lymphocytes, neutrophils and monocytes) in peripheral blood of the study groups (BCG and placebo);
9. Relate the profile of immune response associated with mild and severe forms observed in the study groups (BCG and placebo);
10. Analyze the serum concentration of antibodies and relate it to the immune response;
11. Identify possible antibodies that may confer immunity to new COVID-19 infections;
12. Perform transcriptomic analysis of neutrophilic, lymphocytic and monocytic responses in the study groups (BCG and placebo) involved in the modulation of immune responses in the study groups;
13. Identify predictive biomarkers of infection and/or illness by SARS-CoV-2;
14. Develop a predictive model of infection and/or illness by SARS-CoV-2;
15. Identify socio-demographic factors, life habits, associated diseases and previous vaccinations associated with immune response and the risk of COVID-19 infection.

METHODS:

Study Definitions:

- COVID-19: Positive SARS-Cov-2 test (RT-PCR or serology) + Fever (using self-reported questionnaire) OR at least one sign or symptom of respiratory disease, including cough, shortness of breath, difficulty/respiratory failure (using self-reported questionnaire)

- Severe COVID-19: death, hospitalization or severe non-hospitalized illness, defined as non-walking for 3 or more consecutive days OR inability to work for 3 or more consecutive days

- Asymptomatic infection: Evidence of SARS-CoV-2 infection (by RT-PCR or seroconversion), absence of respiratory disease (using self-reported questionnaire), no evidence of exposure prior to randomization (negative inclusion serology)

- Health Care Worker: For the purposes of this study, in addition to the definition of health professional established in Resolution 218 - 97 - Regulation of Health Professions, the Brazilian Classification of Occupations will be used, and in an expanded way will be included undergraduate students in the area of health sciences who attend the hospital and are in direct contact with patients, as well as administrative technicians who circulate or perform activities in sectors within the hospitals participating in the study.

Study Design:

The project proposes to monitor research participants for 12 months and thus conduct a controlled clinical trial, phase II-B, which compares the occurrence of SARS-CoV-2 infection as well as the time to infection among health care workers receiving BCG vaccine (experimental group) compared to those receiving placebo (control group). Along the clinical trial, participants will also be analyzed in an observational study of two cohorts of health care workers, vaccinated with BCG and not vaccinated. In this part of the study, participants will be analyzed in relation to the inflammatory profile, considering biomarkers predicting infection and evolution to severe form of COVID-19.

Study sites:

The possible participants of this research will be recruited in three hospitals in the city of Rio de Janeiro (Thorax Diseases Institute - Clementino Fraga Filho University Hospital of UFRJ, Pedro Ernesto University Hospital - Piquet Carneiro Outpatients Clinic of UERJ and Central Hospital of the Fire Department of RJ Aristarcho Pessoa - Rio de Janeiro/RJ) and a hospital in the city of Barueri in SP (Hospital Municipal Dr. Francisco Moran - Barueri/SP).

Eligibility criteria:

Health care workers, who perform their work activities in the units participating in the study or not, and who attend patients with probable COVID-19, of both genders, over the age of 18 years and who agree to participate in the study by signing the Free Informed Consent Form (FIC).

***Inclusion criteria**:*

1. Individuals aged 18 ≥, male or female, not infected with SARS-CoV-2
2. Agreement to participate in the study by signing the FIC
3. Not being pregnant (in case of women able to become pregnant)

***Exclusion criteria**:*

1. Professionals with a history of infection confirmed by SARS-CoV-2 or who have already presented a diagnosis of COVID-19 prior to the study
2. Individuals who have not underwent confirmatory tests for COVID-19
3. Breastfeeding
4. Individuals with primary or acquired immunodeficiency
5. Individuals affected by malignant neoplasms
6. Patients treated with high-dose corticosteroids (equivalent to the prednisone dose of 20 mg/day or more) for more than two weeks
7. Patients using other immunosuppressive therapies (antineoplastic chemotherapy, radiotherapy, among others)
8. Individuals with autoimmune diseases
9. Dermatological conditions at the vaccine site or generalized
10. Individuals under treatment for active tuberculosis

11. Individuals with a history of previous tuberculosis treatment

12. Individuals with febrile symptoms (body temperature ≥ 37.5ºC in the last 48h)

13. Participation in other prevention clinical trials for COVID-19

14. Report of vaccination with live microorganism administered in the month prior to randomization

15. Require that, if another vaccination with live microorganism is required, it is administered in the month following randomisation (If the other live vaccine can be administered on the same day, this exclusion criterion does not apply)

16. Known anaphylactic reaction to any ingredient in BCG vaccine

17. Adverse reaction prior to BCG vaccine [significant local reaction (abscess) or suputive lymphadenitis]

18. BCG vaccine administered in the last year

Intervention Plan:

Intervention:

Randomly eligible participants who have provided informed written consent will be recruited. Participants will then be allocated to one of the study regimens in a ratio of 1:1.

1. **Intervention:** BCG vaccine, single dose
2. **Control regimen**: placebo, single dose.

In the BCG clinical trial, participation in the study will last 12 months: during the first 4 months, or for the duration of the recruitment period, participants will receive the randomly designated BCG vaccine or control regimen. After the recruitment period, all study participants should have received BCG or placebo, and both groups will be monitored through clinical and laboratory examination for 12 months of follow-up. Biosafety care to combat COVID-19 adopted in the participating Health Units will also be monitored during the study period.

Comparer:

The natural evolution of SARS-CoV-2 infection in health professionals will be used to evaluate the efficacy of BCG use as preventive therapy, considering the time of positivity of COVID-19 tests and evolution to severe forms of COVID-19.

Allocation and randomization:

The randomization process will be carried out centrally and managed by the team of the Ribeirão Preto School of Medicine of USP (FMRP-USP), under the coordination of Professor Domingos Alves. The process will be performed using the Microsoft Excel® random number generation function. The result will be composed of a randomization table with size appropriate to the sample size expected for the study (1000 participants) and with a ratio of 1:1, which will be integrated to REDCap prior to the beginning of electronic data collection. The creation and validation of the table will be the responsibility of the data manager/project coordinator.

By including a participant in the study, REDCap will consult the existing randomization table on Excel® sequentially to allocated it to one of the groups defined in the study. It is noteworthy that REDCap does not perform, by itself, any form of randomization, which will be based exclusively on the said table in Excel®.

Study visits:

Social networks and electronic media will be used to disseminate the study through folders and calls about the research.

Health professionals from other institutions besides those participating in the study may participate in the research. In this case, they will contact the coordinating center IDT-UFRJ, through telephone contact or email, informing their interest. All follow-up of these participants will be carried out by the coordinating center.

The first evaluation (screening) of the study can be initiated in person or through telephone contact, email or electronic media. If the eligibility criteria are met, the individual will be invited to attend the study center for the signing of the Free Informed Consent and collection of exams that will confirm his or her eligibility or not.

Over the course of six other face-to-face visits [M0 or inclusion, M1 (10 days after the intervention), M3 (90 days after the intervention), M6 (180 days after the intervention), M9 (270 days after the intervention) and M12 (365 days after the intervention) the status of SARS CoV2 infection will be monitored through real-time PCR and serology for after vaccination with BCG or placebo (chart below). The tests will be performed with the purpose of having the diagnosis of the infection either through the RT-PCR technique and/or through the qualitative identification of IgM+IgA and/or IgG antibodies in the detection of infections that occurred asymptomatically and that may have been attenuated by the intervention with the BCG vaccine.

At the time of recruitment HIV serology and BHCG dosage will also be collected for women in order to meet the eligibility criteria. The interferon gamma release assay (IGRA) will be performed only on the inclusion visit or M0 for the purpose of identifying patients previously infected with *Mycobacterium tuberculosis* and detecting interferon gamma (IFN) levels, as well as its role in preventing viral infection, despite knowledge about INF α and β in immunopathogeny against the virus. In the case of participants with previous IGRA reagent and who prove through the exam report will not be submitted to the new collection for this purpose. The other tests will be useful in evaluating the inflammatory response of individuals vaccinated and not vaccinated with BCG and those who became ill or not by COVID-19.

If RT-PCR tests for coronavirus of nasopharyngeal swab, coronavirus serology and/or HIV serology performed during recruitment, generate inconclusive or indeterminate results, participants will be invited to a new collection and confirmation of the result. Participants will only be included with determined negative or non-reactive results for the three exams. In case of participants who present reagent results in HIV serology, they will be referred to the Infectious Diseases service of the study centers for evaluation and follow-up.

In addition to face-to-face visits, participants will receive a telephone contact or a text message by phone via SMS or receive e-mail, the two latter with a link for the participant to access and answer the questionnaire about signs and symptoms of COVID as well as sending a photo of the aspect of the injection site. This will occur weekly until M3, and then monthly seeking information on fever and/or respiratory symptoms and the site of vaccine application. The participant will be asked to send a photo of the vaccine application location in each of these contacts. If the participant signals any respiratory manifestation or fever, they will be instructed to seek the Worker Health Divisions of their units for nasopharyngeal swab to perform RT-PCR for coronavirus. In case of adverse events to the vaccine or placebo patients will be evaluated in person.

If any participant is diagnosed with COVID-19, they will be invited to attend the study for a face-to-face visit once they are asymptomatic and 14 days after the onset of symptoms. In this face-to-face visit, the participant will answer questions from a questionnaire about signs of symptoms of the disease and will be asked to present laboratory or radiological tests performed during COVID-19 infection.

Visits	Evaluations	Exams	Procedures
Screening	Face-to-face assessment of eligibility criteria, application of the Free Informed Consent (FIC)	Nasopharyngeal swab RT-PCR, Antibody levels anti-SARS-Cov-2, BHCG dosage and HIV serology	Invitation to participate in the study, application of the FIC, collection of clinical samples (nasopharyngeal swab and blood).
Inclusion or M0 **0 day of vaccination (BCG or placebo)**	Face-to-face assessment with confirmation of eligibility, clinical inclusion visit, collection of exams and dose of BCG or placebo	IGRA; Complete blood count, lipidogram, albumin, HbGlic, DHL, CPR, ESR, D-Dimer; and biomarkers*.	Nursing and medical consultation. Collection of clinical samples (blood). Application of preventive treatment.
S1 - S12 **7-90 days of vaccination**	Telephone and/or SMS contact, and/or weekly email and sending photo of the injection site	No exams	Fever questionnaires and/or respiratory signs and symptoms and photo submission of the injection site
M1 **10 days of vaccination**	Face-to-face visit to the laboratory for blood collection for biomarkers	Antibody levels Anti-SARS-Cov-2; Complete blood count, lipidogram, albumin, HbGlic, DHL, CPR, ESR, D-Dimer; and biomarkers*.	Collection of clinical samples (blood).
M3 **90 days of vaccination (±14 days)**	Face-to-face clinical visit for follow-up and blood collection for serology	Antibody levels Anti-SARS-Cov-2	Nursing and/or medical consultation. Collection of clinical samples (blood).
M4/M5 **120/150 days of vaccination (±14 days)**	Telephone contact and/or SMS, and/or weekly email	No exams	Fever questionnaires and/or respiratory signs and symptoms
M6 **180 days of vaccination (±14 days)**	Face-to-face visit to the laboratory for blood collection for serology	Antibody levels anti-SARS-Cov-2.	Collection of clinical samples (blood).
M7/M8	Telephone contact and/or SMS, and/or weekly email	No exams	Fever questionnaires and/or respiratory signs and symptoms

210-240 days of vaccination (±14 days)			
M9 **270 days of vaccination (±14 days)**	Face-to-face visit to the laboratory for blood collection for serology	Antibody levels Anti-SARS-Cov-2	Collection of clinical samples (blood).
M10/M11 **300-330 days of vaccination (±14 days)**	Telephone contact and/or SMS, and/or weekly email	No exams	Fever questionnaires and/or respiratory signs and symptoms
M12 **365 days of vaccination (±14 days)**	Face-to-face clinical visit to close the study and for blood collection for serology	Antibody levels Anti-SARS-Cov-2	Nursing consultation. Collection of clinical samples (blood).
Fever and/or respiratory signs and symptoms	Face-to-face visit to the Worker's Health sector of its study center to collect exams	Nasopharyngeal swab RT-PCR	Clinical samples collection (swab and blood).
	Face-to-face clinical visit to the study center since asymptomatic and 14 – 30 days after the onset of symptoms	Antibody levels Anti-SARS-Cov-2; Complete blood count, lipidogram, albumin, HbGlic, DHL, CPR, VHS, D-Dimer and biomarkers*.	Nursing or medical consultation. Copy of the tests performed during COVID-19 infection. Collection of clinical samples (blood).

Subtitles: M0: Moment zero; S1-S12: Weeks 1 to 12; M1: Moment 1; M3: Month 3; M4: Month 4, M5: Month 5; M6: Month 6; M7: Month 7; M8: Month 8; M9: Month 9, M10: Month 10; M11: Month 11; M12: Month 12; *Clinical Analyses and Biomarkers: They will be performed only in hucff-idt ufrj, hupe-ppc uerj and hcb aristarcho pessoa centers.

Storage of specimens during study visits:

The nasopharyngeal swab will be obtained for the realization of the real time – polymerase chain reation (RT-PCR) for COVID-19. The collection will be performed using the technique described in the Manual of the Ministry of Health. The collection should be performed with the rubbing of the swab in the posterior region of the nasal caress trying to obtain some of the mucosal cells. The swab will be stored in 15ml tubes containing DMEM + Fetal Bovine Serum 10% + L-Glutamine Solution–Penicillin–Streptomycin.

After collection, the specimen will be packed in a refrigerator of specimens from the research center with temperature control between 2 - 8 °C. The exam kit batch number must be registered in a specific form.

Blood collection will comply with the guidelines of the Ministry of Health in the Manual Techniques for Blood Collection (2001) for the performance of blood count, lipidogram, albumin, LDH, ESR, C-reactive protein, D-dimer and dosage of biomarkers described in the study protocol. The collected material will be distributed in 11 blood tubes [1 SST tube, 2 EDTA tubes – purple cap (4.5ml/each), 1 serum tube - red cap (4 ml/each), 1 gel activator tube - yellow cap (4 ml/each), 2 tubes with soric heparin - green cap (8 ml/each) and 1 Tempus tube – blue cap (3 ml/each). During the screening visit, blood will be collected for BHCG dosage and HIV serology. In the inclusion visit the material will also be sent for IGRA [4 tubes Kit IGRA (1ml/each)].

After collection, the specimen will be packed in a refrigerator of specimens from the research center with temperature control between 2 - 8 °C.

All specimens will be delivered to their destination laboratories, transported in thermal boxes maintaining the necessary refrigeration and obeying the criteria of storage and maintenance of the viability of the collected material.

Procedure for applying BCG vaccine or placebo:

BCG vaccine:

The application of BCG vaccine as well as its storage and the materials used for application will follow the standards of the Manual of Standards and Procedures for Vaccination of the Ministry of Health (Brazil, 2014). As standardized in this Manual, the vaccine will be applied intradermally to the lower insertion of the deltoid muscle of the right arm, except contraindications or impossibilities. Other details are described in the standard operating manual (MOP) of the study. After the intervention, participants will be under observation for 20 minutes in the study center waiting room and will be released after the period as long as they do not present adverse events.

Placebo:

A 0.1 ml of 0.9% NaCl saline solution applied intradermally in the lower insertion of the deltoid muscle of the right arm will be used as placebo, except contraindications or

impossibilities. Other details are described in the standard operating manual (MOP) of the study. After the intervention, participants will be under observation for 20 minutes in the study center waiting room and will be released after the period as long as they do not present adverse events.

Blinding:

The study participants as well as the researchers, coordinators, nurses, physicians and digitizers of the study will be blind to the allocation in the experimental or control groups. Only nursing technicians who will apply the vaccine or placebo will be aware of which group the patient will be allocated to. This professional will not follow up on the clinical follow-up or outcome of the study participant. Other details are described in the standard operating manual (MOP) of the study.

Reaction to BCG vaccine and placebo:

The BCG vaccine known to generate a local inflammatory response that lasts about 12 weeks and will be monitored throughout the study. The reactions are described below and will not be considered as adverse events:

- From the first to the second week: reddish macula lasting from 5 mm to 15 mm in diameter.
- From the third to the fourth week: pustule that forms with the softening of the center of the lesion, followed by the appearance of crust.
- From the fourth to the fifth week: ulcer with 4 mm to 10 mm in diameter.
- From the 6th to the 12th week: scar with 4 mm to 7 mm in diameter, found in about 95% of those vaccinated.

Although the response to placebo did not cause any local vaccine reaction, which would question its use, we believe that if we did not use any intervention in the control group, bias could occur, in view of the possible reduction of precautionary measures and care in the use of personal protective equipment (PPE) in the experimental group, believing that they were "protected" by a vaccine, although its efficacy in this situation was not proven. On the other hand, those not vaccinated would be more careful in relation to hygiene measures and use of personal protection equipment. Using placebo, we believe that we match the usual prevention measures to COVID-19 in the study population at least in the

first month of follow-up, a period prior to the onset of the lesions expected by BCG vaccination.

Adverse events:

The definition for an adverse event described in the Manual for Notification of Adverse Events and Safety Monitoring in Clinical Trials (Anvisa, 2016) will be used as a definition for an adverse event (AE): "any adverse medical occurrence in a patient or clinical trial participant to whom a pharmaceutical product has been administered and who does not necessarily have a causal relationship to treatment. As a result, an AEs can be any unfavorable and unintentional sign, symptom, or disease (including results outside the reference range), associated with the use of a product under investigation, whether related to it or not. A serious AE shall be considered to have one resulting in any adverse experience with medicinal products, biological products or devices occurring at any dose and resulting in any of the following outcomes: a) death; b) threat to life; c) persistent or significant disability/disability; d) requires hospitalization or prolongs hospitalization; e) congenital anomaly or birth defect; f) any suspected transmission of infectious agent by means of a drug or; g) clinically significant event."

Adverse events that do not fall within the definition of severe AE (definition above) will be graduated according to Heath Surveillence Agency of Brazil (ANVISA) 2016:

- Light Level: Problem present less than 25% of the time, with an intensity that a person can tolerate and that rarely happened in the last 30 days;
- Moderate Level: A problem that is present less than 50% of the time, with an intensity that interferes with the day-to-day of people and that has occurred occasionally in the last 30 days.
- Severe Level: Problem that is present in more than 50% of the time, with an intensity that partially alters the day-to-day of the person and that has happened frequently in the last 30 days.
- Complete involvement: It means that a problem that is present in more than 95% of the time, with an intensity that completely alters the day-to-day of the person and that has occurred every day for the last 30 days.
- Unspecific: Means that there is not enough information to specify the intensity.

- Not applicable: It means that it is inappropriate to use a gradation (e.g. menstrual functions).

All AEs will be notified to the local Ethics Committe (EC) by the study coordinator at each centre using its own form (MOP Annex 3.3). The AEs should also be reported to the coordination of the proposing institution. The AE notification form as well as proof of sending the notification to the local EC must be sent by e-mail to the coordinator of the proposing institution no later than 7 days after notification.

In the case of adverse events related to BCG vaccine (ulcer with diameter greater than 1 cm, cold subcutaneous abscess, hot subcutaneous abscess, granuloma, regional lymphadenopathy not suppurated greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar or lupoid reaction), the coordinator of the proposing institution will report to the National Immunization Program of the Ministry of Health.

With regard to placebo, a minimum volume of saline solution applied intradermally will be used. The saline solution itself does not have any immunogenic component and does not cause any adverse events. It is the same solution used for intravenous or oral hydration and for dilution of medicinal products used intravenously. After intradermal application, mild pain may occur and after a few days local redness. Local signs of infection are not expected and the same vaccination standards and protocols established by the National Immunization Program will be used.

Serious adverse events:

Serious adverse events (SAE) should be reported immediately to the coordination of the proposing institution through telephone contact (described in MOP). From this notification, the SAE must be reported to ANVISA within a maximum of 24 hours by the coordinator of the proposing institution through the agency's electronic portal (Serious Adverse Events Notification Form in Clinical Trials available on the ANVISA Electronic Portal > Medicines > Clinical Research > Adverse Events > Form for Notification of Serious Adverse Events in Clinical Trials - Notification EC).

All serious adverse events suspected to have been caused by the proposed experimental treatment, including deaths will be reported to National Ethics Committe, through notification and within 24 hours.

Participants will receive all care and will be guaranteed assistance.

Both the vaccine and placebo will be applied using disposable materials and patients will have possible adverse events recorded in their own form and notified if they occur (MOP Annex 3.3). In addition, participants will be guaranteed assistance if they present any adverse event.

RISKS:

The safety of BCG revaccination among health professionals will be evaluated in order to describe possible injection site reactions and other adverse events.

In addition, we will minimize the occurrence of AE, excluding immunosuppressed individuals or using concomitant medications that impact immune responses.

Local and systemic AEs will be evaluated at relevant times through medical visits, for example in the first 20 minutes after BCG injections and in later visits, either in person or by telephone contact. The AEs will be noted and graduated according to the ANVISA Manual.

Adverse events to the BCG vaccine, more frequent have been abscess and lymphadenopathy, with higher reports for men than for women. The rates of AEs per 10,000 distributed doses were from 11.6 to 15.4, respectively. Unsuppurated regional lymphadenopathy greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar, or lupoid reaction may occur. The AEs will be monitored and handled throughout the follow-up visits.

The research centers will also be open and available for extra views in case of adverse events. The manifestations will be recorded in the data collection instruments and will be treated and monitored in hospitals linked to the research centers. Similarly, the events will be notified to the National Immunization Program of the Ministry of Health, informing the batch of the vaccine applied as well as the event that occurred. Participants will receive all care and will be guaranteed assistance.

BENEFITS:

The results of this study may help define a form of prevention of COVID-19 for health professionals and in the future in the population as a whole.

LABORATORY PROCEDURES:

Blood collection:

Conventional examinations will be called those performed by the Clinical Pathology laboratories contracted for the purpose of the study.

These are: blood count, erythrocyte sedimentation rate (ESR), lactate dehydrogenase (DHL), total cholesterol and fractions and triglycerides, D-dimer, reactive C protein (RCP), albumin, ferritin, iron, glycated hemoglobin. For this purpose, 1 EDTA tube – purple cap and 1 serum tube (red cap) should be delivered for laboratory analysis.

Polymerase chain reaction with real-time reverse transcription:

The presence of SARS-CoV-2 in respiratory samples will be detected by real-time amplification by RT-PCR from the open reading chart SARS-CoV-2 1ab (ORF1ab), nucleocapsid protein (NP) gene fragments using kits provided by IDT USA. The conditions for amplifications will be 50 °C for 15 minutes, 95 ° C for 3 minutes, followed by 45 cycles of 95 ° C for 15 if 60 ° C for 30 s. When two targets (ORF1ab, NP) test positive by specific real-time RT-PCR, the case will be considered laboratory confirmed. A cycle threshold value (Ct value) less than 37 will be defined as a positive test and a Ct value of 40 or more has been defined as a negative test. An average load, defined as a Ct value of 37 to less than 40, will require confirmation per new test.

Serological test for COVID-19

The Vircell serological test will ® to define the inclusion or not of health professionals in the study. Vircell® reveals the presence of IgG and IgM/IgA against viral antigens as well as their titration, facilitating the monitoring of absolute antibody values.

Immunoassays

Seros will be obtained from the health professionals enrolled in the study. Two cohorts will be considered: health professionals vaccinated with BCG (n=500) and health professionals not vaccinated with BCG (n=500).

The biomarkers evaluated will be cit-H3, alpha 1 AT, D-dimer, LDH and CPR. The analyzed data will be related to the blood count information, according to the scheme: M0, M1, M3 and M12.

Inflammatory profile and population characterization of neutrophils and T lymphocytes

A panel of surface and intracellular markers more related to exacerbated inflammation will be used, aiming to study the profile of T lymphocytes, monocytes and neutrophils.

Cell profiles will be compared to the occurrence or not of SARS-CoV-2 infection in both cohorts.

These analyses will be performed at the defined times of collection and evaluation of the study groups. The markers to be studied in cell populations will be:

Lymphocyte T: CD3, CD4, CD119, IL-12Rβ2, IFN-γ, TNF-α, IL-4, IL-10, IL-13, IL-21, IL-1RI, IL-6Rα, IL-17A, IL-17F, IL-22, FoxP3, CD25, Galectin-3, IL-35, TGF-β.

Neutrophil: CCL22, CD169, CD68, ZAP70, CD11b, CD11c, LDH, CD64, IL-17.

Monocytes: CD14, CD11c, HLA-DR, CD45, IP-10, MIP-1α, MIF.

Inflammatory and metabolic profiles will be measured by luminex, using serum from patients for the panel: IFN-α2, IFN-γ, IL-10, -12 p40, -12 p70, -13, -15, -1 Ra, -1α, -1β, -2, -4, -6, -8, -23, -17, -18, -22, MIP- 1 α, MIP-1β, TNF-α, VEGF-A, Adiponectin, Hepicidine, Insulin, MMP-1, -2, -8, -9, MIF, Glucagon, Ghrelin, leptin, CD163s, Galectin-1 and -3, Vitamin DIN Protein , PCR, albumin, histona citrunilada H3, alpha1-anti-trypsin, P-selectin, D-dummer, PSGL-1, tPA, CD40L, PAI-1, Tissue Factor IX, CXCL8 (IL-8).

Transcriptoma

RNA obtained from peripheral blood cells will be used to evaluate the signaling pathways of monocytes and neutrophils in both study groups. RNA sequencing (RNASeq) will be used to evaluate the profiles, according to the manufacturer's instruction (Illumina).

Laboratory procedures to be performed at each research center:

Laboratory procedures will be different according to the research center where the participant is included, as follows:

	Recrutamento	**Inclusão ou M0**	**M1**	**M3**	**M6**	**M9**	**M12**	**COVID suspeito ou confirmado**	**14 – 30 dias após COVID-19 suspeito ou confirmado**
HUCFF-IDT UFRJ	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA, análises clínicas, biomarcadores	Sorologia Coronavírus, análises clínicas, biomarcadores	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus, análises clínicas, biomarcadores
HUPE-PPC UERJ	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA, análises clínicas, biomarcadores	Sorologia Coronavírus, análises clínicas, biomarcadores	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus, análises clínicas, biomarcadores
HCCBAP RJ	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA, análises clínicas, biomarcadores	Sorologia Coronavírus, análises clínicas, biomarcadores	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus, análises clínicas, biomarcadores (Ctrl)
HMFM Barueri SP	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus

DATA COLLECTION AND MONITORING PLAN:

The researchers involved in this protocol will use the forms as source documents (SD). The centers will record the data in the SDs when the data is generated (example: during the participant's visit or during telephone contact). The team will use the REDCap (Electronic Survey Data Capture) system for electronic data capture and data management and its templates will be called Case Report Forms (CRF). A copy of the REDCap database will reside on a secure FMRP-USP server that has all physical, technical, and administrative controls. A second copy will be stored on the REDCap server for TB network data. We will use the group of data management researchers from the Faculty of Medicine of Ribeirão Preto-USP. Any professional of the team who is delegated to access the data system, either to access or to do quality control of the data, will have individual access with basic security requirements. The data will be retrieved from the centers so that no center has access to data from other centers. However, the study's lead investigator will have access to data from all centers. The team responsible for REDCap will manage access to the REDCap system, creating, suspending and exhaling user logins as needed. The centers of Brazil will be responsible for informing the team responsible for data management in advance when a professional is disconnected from the protocol, and then their respective access to the system will be canceled. The list of users who will have access to the protocol REDCap will be reviewed monthly.

As recently, the release of the REDCap software license (https://projectredcap.org/) on behalf of REDE-TB was obtained, it will become part of the REDCap Consortium, a network of all partners that make an instance of the software available. The software was installed in the cloud computing environment of the University of São Paulo. REDCap supports multiple research projects in parallel. Therefore, once installed and configured, it can be used repeatedly for an indefinite period. We will use an intuitive and user-friendly KoBoToolbox (https://www.kobotoolbox.org/) interface as it also enables online and offline data collection through computers and mobile devices. It can be used together with REDCap. The cloud computing environment of the University of São Paulo, called interNuvem (https://internuvem.usp.br), will be used to create virtual machines to instantiate the technological resources needed to execute the project, such as database servers, REDCap, Web servers, among others. As a way to manage the features of the REDCap and KoboToolbox software suite, the data collection forms, initially defined on paper, will be computerized. Offline data collection can be done through the mobile app (Android, iOS). In this case, the data must be transmitted to the server when internet

connectivity is established. The application will be made in the Elixir programming language, which was created on top of erlang, and so it is a functional language. Within the Project there are three main modules are them: Redcap; XLSX Redcap Form Decode and Redcap.Encoder.

The first module is responsible for reading the files used in the translation of the forms provided by KoboToolbox, in XLS format, to Redcap. The main operations involved in this process are migration of the form structure and the collected data. During the first operation, a function is responsible for reading the xls file and uses the XLSX Redcap Form Decode module, to transform each line of the file into an Elixir structure. After having each row schemated in these structures, it's time to call Redcap.Encoder that will transform this structure received from the previous module and transform into a new data structure by translating each of the rows into its data dictionary equivalents. When all lines are ready the main module uses this structure and "writes" in a data dictionary for Redcap.

In the second operation everything happens in the same Redcap module. In it the data files are read and with the help of a file that I called "data_guide" it reorganizes the data the way Redcap asks for import. It also replaces the headers that are named after the xlsform data for the names that were registered in Redcap. This is done through "data_guide", which is a file that has on one side the column name in xlsform and the name of that information on the Redcap platform on the other. In addition to replacing headers and reorganizing columns, the data also goes through a normalization to be in accordance with what Redcap accepts. For example all "True" and "False" that are put in Ona for multiple selections have to be replaced by 1 and 0 respectively, otherwise Redcap does not accept. After these steps the final structure is written in csv for import.

Specific protocols for electronic quality monitoring and tracking will be performed by a data manager routinely, in order to detect fill errors, inconsistent or missing data, thus ensuring quality control.

An independent study monitor, appointed by the lead researcher, will be responsible for monitoring data quality by standard test operating procedures. Based on the monitoring plan, the field visit and audit will be carried out at different stages. All records of participants, DFs and other source documents of the patients recruited in this study will be made available for analysis by the monitors. A meeting of researchers from each site will take place monthly, via the web-based remote conferencing system, to share the progress of the study and discuss c problems encountered while conducting the test.

During the conduct of the research, any critical modifications in the protocol will be informed to the principal investigator, to the study record and, if relevant, to the study participants.

Sample storage plan:

Nasophayngeal swab and peripheral blood specimens collected will be transported to specific laboratories. Specimens may be stored to facilitate the repetition of the test in case of indeterminate results or discordant cases in molecular laboratory tests or not for SARS-CoV-2. Stored clinical specimens will be labeled with a study identifier and not with personal identifiers and will be used only for the purposes of this study.

According to CNS Resolution No. 441 of 2011 (Art. 1) and Ordinance MS No. 2,201 of 2011 (Art. 3), any organized collection of human biological material collected for scientific research purposes, including samples intended for routine examinations in a clinical trial (e.g., blood count and renal function) are considered as constituents of a biorepository or biobank. The biological material will be used as provided for in the research protocol, with no further future analyses different from those foreseen. If there is a proposal for future use of the stored samples, approval in the institute or national EC system will be required and a new protocol and FIC will be submitted so that each participant can choose whether or not to allow the use of the samples for future studies.

Statistical Analysis Plan:

The primary efficacy analysis will compare the cumulative incidence of SARS-CoV-2 infection and the cumulative incidence of severe forms of COVID-19.

These analyses will occur when the last enrolled research subject has completed 30 days and 6 months of preventive treatment and will be performed using the results of COVID-19 diagnostic tests, as well as the data available in participating hospitals and national electronic notification systems of COVID-19. A modified treatment intention analysis (MITT) and protocol will be performed. The safety and tolerability analysis will consist of all patients who have been randomized and received a dose of the vaccine or placebo. Based on serum concentrations of the immune response, we will calculate

certain immunoinflammatory variables related to the use of BCG versus placebo among subjects infected and not infected by COVID-19.

The intervention and control groups will be compared at the time of inclusion in the study in relation to sociodemographic, clinical, radiological and biochemical imaging variables, in order to evaluate the adequacy of the randomization process. Multivariate analyses using logistic regression or Poisson models with robust variance will be used to evaluate the effect of BCG vaccine on primary and secondary outcomes. Associations will be expressed in the form of risk ratios and respective 95% confidence intervals. Data imputation may be required and the random forest system will be used. Statistical software R, version 10.0.

Sample size:

It is estimated that it will take 500 individuals in each of the comparison groups to ensure a statistical power of 85% for the detection of a risk difference of 5%, considering that the control group will have an accumulated incidence of SARS-CoV-2 infection of 10% after 6 months of follow-up, for a type I error of 5%.

With 500 individuals in each of the comparison groups the study will have a statistical power of approximately 75% for the detection of a risk difference of 2%, considering that the control group will have an accumulated incidence of more severe forms of COVID-19 of 2.5% after 6 months of follow-up, for a type I error of 5%.

We will also use for the two outcomes (below) the sequential design the groups are followed up until there is a group with clear benefits or that both groups do not present difference. This design is used when the results need to be known very quickly. If there is a large difference between the treatments, then this study will be performed in a shorter time when compared to the parallel trial. The main ethical advantage is that if one treatment shows a superiority over the other, the study can already be stopped. However, the interim analyses will be previously planned, in such a way that the calculation of sample size allows the multiplicity of the tests. The meaning of p-values in sequential analyses also changes, because when using sequential analyses, more than one analysis is performed, and the typical definition of a p-value as data "at least as extreme" as observed needs to be reset. One solution is to sort the p-values of a series of sequential tests based on the downtime and at what time the test statistic was in a certain appearance, known as ordering.

OUTCOMES:

Primary Outcome

1. Compare the cumulative incidence of SARS-CoV-2 infection

It is estimated that it will take 500 individuals in each of the comparison groups to ensure a statistical power of 85% for the detection of a risk difference of 5%, considering that the control group will have an accumulated incidence of SARS-CoV-2 infection of 10% after 6 months of follow-up, for a type I error of 5%.

2. Compare the cumulative incidence of severe forms of COVID-19

With 500 individuals in each of the comparison groups the study will have a statistical power of approximately 75% for the detection of a risk difference of 2%, considering that the control group will have an accumulated incidence of more severe forms of COVID-19 of 2.5% after 6 months of follow-up, for a type I error of 5%.

We will also use for the two outcomes the sequential design the groups are followed up until there is a group with clear benefits or that both groups do not present difference. This design is used when the results need to be known very quickly. If there is a large difference between the treatments, then this study will be performed in a shorter time when compared to the parallel trial. The main ethical advantage is that if one treatment shows a superiority over the other, the study can already be stopped. However, the interim analyses will be previously planned, in such a way that the calculation of sample size allows the multiplicity of the tests. The meaning of p-values in sequential analyses also changes, because when using sequential analyses, more than one analysis is performed, and the typical definition of a p-value as data "at least as extreme" as observed needs to be reset. One solution is to sort the p-values of a series of sequential tests based on the downtime and at what time the test statistic was in a certain appearance, known as ordering.

3. Assess the BCG vaccine-mediated immune response in health care professionals

SARS-CoV2 infection is estimated to occur in approximately 10% of BCG-vaccinated healthcare professionals (experimental group) and in 30% of non-BCG-vaccinated healthcare professionals (placebo group)

In this scenario, with the bearing of 500 health professionals in each arm, SARS-CoV2 infection is expected to be detected in 50 BCG-vaccinated professionals and 150 professionals not vaccinated with BCG. For each subgroup (vaccinated and not

vaccinated with BCG) it will be possible to identify the predictive biomarkers of infection by comparing infected professionals with those who were not infected with SARS-CoV2.

INTERIM ANALYSIS PLAN:

In relation to the effect of BCG vaccine administration on the cumulative incidence of SARS-CoV-2 infection or on the severe forms of COVID-19, a single intermediate analysis will be performed when the number of volunteers included has reached half of the total sample scheduled to verify whether the evidence would already be conclusive and would allow the study to be interrupted. For type I error protection, intermediate analysis will use a Z value of 2.78 (Chow & Liu, 2004).

The analyses that will be performed in the intermediate analysis will be restricted to the following outcomes: accumulated incidence of SARS-CoV-2 infection, cumulative incidence of severe forms of COVID-19 and proportion of serious adverse events.

The following rules will be used to indicate early completion of the study after intermediate analysis:

• Significant difference in the effect of BCG judged by comparing the proportions of patients who develop SARS-CoV-2 infection or severe forms of COVID-19 in each arm of the study;

• If the effect of BCG is similar between groups and there is a significant difference in the cumulative proportion of serious adverse events;

The results will be evaluated by the Data and Safety Monitoring Committee, which will express the need to continue the study, based on the vaccine's performance in terms of the incidences of SARS-CoV-2 infection or severe forms of COVID-19 and adverse events.

Independent Data and Safety Monitoring Committee:

The Data and Safety Monitoring Committee (DSMC) will be composed of three members not linked to participating institutions, in accordance with the Operational Guidelines for the Establishment and Functioning of Data Monitoring and Safety Committees of the Ministry of Health (2008).

This committee will be responsible for reviewing the protocol and progress of the study in all centers, with real-time meetings by videoconference, as detailed below, and will consist of:

Denise Rossato - Physician - Professor, Faculty of Medicine, Federal University of Rio Grande do Sul

Ronir Raggio - Statistician - Institute of Collective Health Studies of the Federal University of Rio de Janeiro
Silvana Spíndola - Physician - Professor, Faculty of Medicine, Federal University of Minas Gerais

An early safety assessment will be carried out when the first 100 participants (approximately 50 per arm) are included. Security data will be reviewed by a DSMC member or someone designated by a DSMC member. In addition, the Committee will review the progress of the study after the inclusion of every 100 additional participants through real-time meetings by videoconference.

CMDS activity plan:

Time of study	**Activity**	**Way**
Before the start of the study	Protocol and MOP review	Real-time video conferencing scheduling
During the study	Early safety assessment after inclusion of the first 100 participants	Real-time video conferencing scheduling
	Evaluation of study progression per 100 participants included	Real-time video conferencing scheduling
	Adverse event analysis	Real-time video conferencing scheduling every 2 weeks
	Analysis of serious adverse events	Extraordinary meeting via real-time videoconferencing
	Intermediate analysis after the inclusion of 500 participants	Real-time video conferencing scheduling
At the close of the study	Evaluation of the conduct of the study and its results	Real-time video conferencing scheduling

Plan for monitoring and analysis of adverse events:

The definition for an adverse event described in the Manual for Notification of Adverse Events and Safety Monitoring in Clinical Trials (ANVISA, 2016) will be used as a

definition for an adverse event: "any adverse medical occurrence in a patient or clinical trial participant to whom a pharmaceutical product has been administered and who does not necessarily have a causal relationship to treatment. As a result, an AEs can be any unfavorable and unintentional sign, symptom, or disease (including results outside the reference range), associated with the use of a product under investigation, whether related to it or not. A serious AE shall be considered to have one resulting in any adverse experience with medicinal products, biological products or devices occurring at any dose and resulting in any of the following outcomes: a) death; b) threat to life; c) persistent or significant disability/disability; d) requires hospitalization or prolongs hospitalization; e) congenital anomaly or birth defect; f) any suspected transmission of infectious agent by means of a drug or; g) clinically significant event."

All AEs will be notified to the local EC by the study coordinator at each centre using their own form. AEs shall also be reported to the coordination of the proposing institution no later than 7 days after notification of the AE.

In the case of adverse events related to BCG vaccine (ulcer with diameter greater than 1 cm, cold subcutaneous abscess, hot subcutaneous abscess, granuloma, regional lymphadenopathy not suppurated greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar or lupoid reaction), the coordination of the proposing institution should be notified to the National Immunization Program of the Ministry of Health.

Serious adverse events (SAE) should be reported immediately to the coordination of the Proposing Institution through telephone contact. From this notification, the SAE must be reported to ANVISA within a maximum of 24 hours by the coordinator of the proposing institution through the agency's electronic portal (Serious Adverse Events Notification Form in Clinical Trials available on the Anvisa Electronic Portal > Medicines > Clinical Research > Adverse Events > Form for Notification of Serious Adverse Events in Clinical Trials - Notification EC).

All serious adverse events suspected to have been caused by the proposed experimental treatment, including deaths will be reported to National EC, through notification and within 24 hours.

There will be a review of the AE data every 2 weeks. If adverse events are unexpected or not previously described, case review will be requested through a meeting of the data monitoring and security committee, which should take place in real time by

videoconference. Serious adverse events will be immediately flagged to the DSMC for extraordinary analysis also by real-time videoconferencing.

Criteria for discontinuation of the study for safety reasons:

No formal rule will be adopted to interrupt the study for safety reasons. The DSMC may recommend stopping the study, based on the review of interval safety results. The study can only be canceled or discontinued after analysis of the reasons for discontinuity by the institution's or National Ethics Committe.

Criteria for discontinuation of experimental treatment in the participants:

No formal rule will be adopted to discontinuation of experimental treatment in the study participants. The DSMC may recommend this study interruption, based on the review of interval results as described above. Nevertheless, this decision can only be effected after evaluation and approval by the institution's National EC.

QUALITY MANAGEMENT:

All studies will be conducted in accordance with the ICH-GCP Guidelines (ICH-GCP E6 R2), as far as possible in the research environment, as well as all national legal and regulatory requirements (as applicable).

BUDGET:

Cost	**Value**
Legal Entity	R$84,000.00
Scholarship	R$160,800.00
Inputs	R$297,188.57
Other	R$43,571.43
Capital	R$14.440,00
Total	R$600,000.00

SCHEDULE:

The schedule presented below is a schedule, and the study should be started only after approval of the CEP.

Moment	**Beginning**	**End**
Submission of amendment to Ethics Committe	17/11/2020	01/12/2020
Recruitment/Inclusion/Intervention	30/10/2020	01/02/2021
Follow-up of patients	01/10/2020	01/02/2022
Data analysis	01/02/2022	01/04/2022
Reporting/Results	01/02/2022	01/07/2022

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